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APR 79 J A FORET, J R BARRY DACW39-74-C-0074

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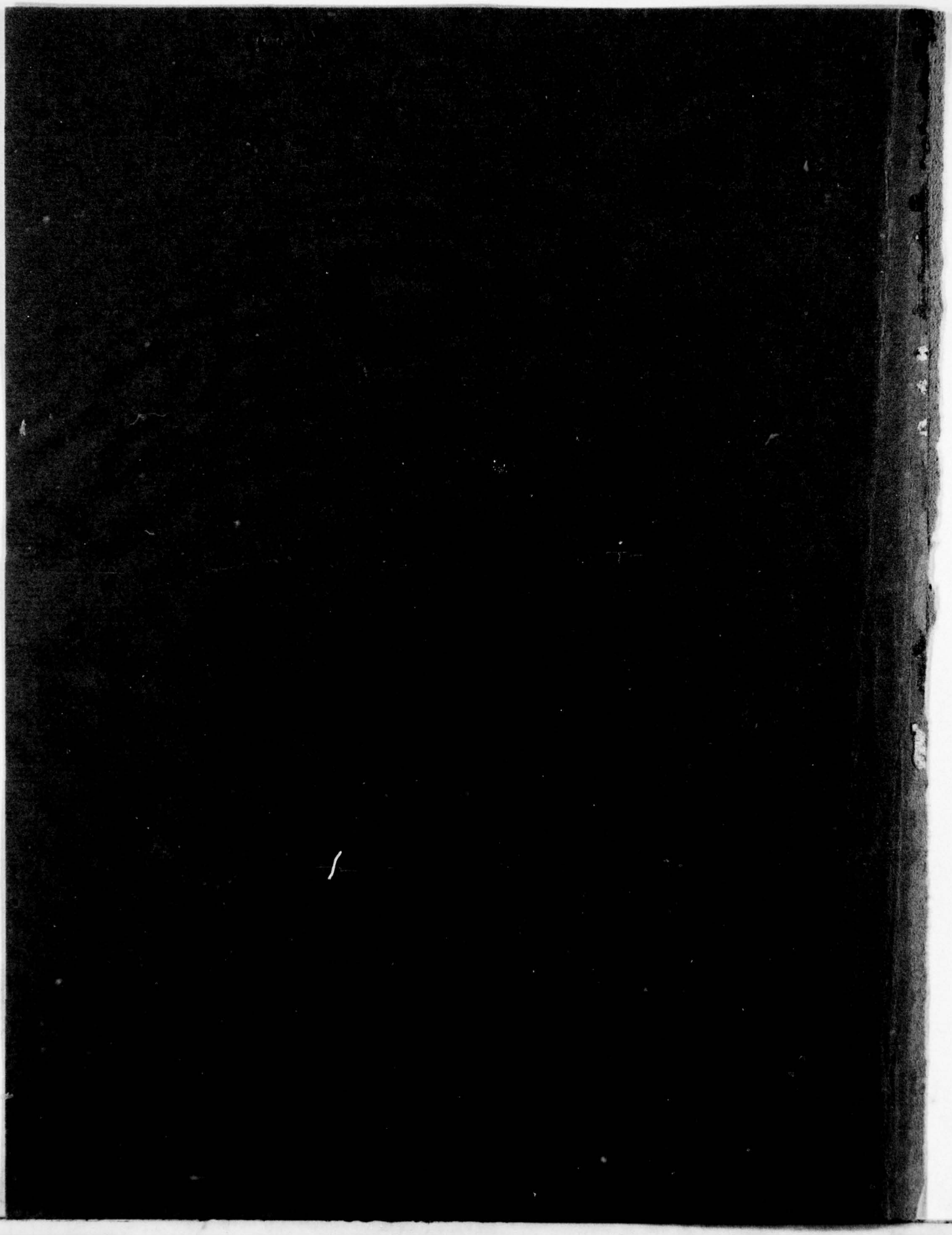

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The waterhyacinth (*Eichhornia crassipes*) is a problem aquatic plant in the slow-moving, quiescent waters of the Gulf Coastal States. To institute control, programs utilizing the herbicide 2,4-D were initiated. These have proven to be the most practical and effective control measures available to date. This project was undertaken to provide data that would facilitate the registration of 2,4-D DMA by the Environmental Protection Agency (EPA) as an aquatic herbicide for control of waterhyacinth in slow-moving water. The experiment was designed to provide data on 2,4-D residues within the application site.

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PREFACE

The study reported herein was performed under Contract No. DACW39-74-C-0074 with the Department of Plant Industry of the University of Southwestern Louisiana, Lafayette, Louisiana, for the Office, Chief of Engineers. The study was conducted and the report was prepared by Drs. James A. Foret and J. Robert Barry of the University of Southwestern Louisiana.

The research was monitored by the U. S. Army Engineer Waterways Experiment Station (WES). The study was conducted under the general supervision of Messrs. W. G. Shockley, Chief, Mobility and Environmental Systems Laboratory, B. O. Benn, Chief, Environmental Systems Division, and J. L. Decell, Chief, Aquatic Plant Research Branch (APRB). APRB is now part of the recently organized Environmental Laboratory of which Dr. John Harrison is Chief.

Directors of the WES during this study and preparation of this report were COL G. H. Hilt, CE, and COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

CONTENTS

TIME COURSE STUDIES ON 2,4-D AMINE RESIDUES IN SLOW-MOVING WATERS

PART I: INTRODUCTION

Background

1. The waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) was introduced into Louisiana in 1884 and by 1950 had infested an estimated 10 to 15 percent of the 810,000 ha of lakes, ponds, canals, and rivers in the state.[1] In Florida, the growth and spread of this pest was not as rapid, but by 1970 the same degree of infestation was evident. Waterhyacinth is currently a problem in most of the southern United States.

2. Removal of waterhyacinths from navigable waters in the United States was first authorized by Congress in the River and Harbor Act of 1899. The resources required for this control program could not be fully validated from channel control operations, so the Congress in 1958 authorized a 5-year pilot project for progressive control and eradication of waterhyacinth, alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb), and other obnoxious aquatic plants in navigable waters, tributary streams, connecting channels, and other allied waters in the coastal states from North Carolina to Texas. The project was initiated in the combined interests of navigation, flood control, agriculture, drainage, public health, fish and wildlife conservation, and related purposes.

3. Approximately 25 years ago, the waterhyacinth control program in Louisiana initiated the use of 2,4-D* as a chemical means of control. The waterhyacinth is highly sensitive to 2,4-D, and good control resulted during early control operations. Because massive infestations of this weed were seriously impeding navigation, drainage, irrigation, and sports and recreation, an all-out control program was developed by the U. S. Army Engineer District, New Orleans.

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* 2,4-dichlorophenoxyacetic acid.

4. Spray programs using salts of 2,4-D were adopted in all of the southeastern states over the past 15 to 20 years to control this ever-increasing aquatic weed. The fact that large areas of water were being sprayed with 2,4-D to control the waterhyacinth led to studies of the fate of this herbicide in water and sediment within the treated areas.[2,3] Crosby and Tutass found that 2,4-D decomposes rapidly in the presence of water and ultraviolet light or sunlight.[4] Soil microorganisms also convert 2,4-D to 2,4-DCP which subsequently undergoes ring hydroxylation. It has also been suggested that microorganisms cleave the ring structure in the degradation process.[5,6]

5. Experimental studies of 2,4-D formulations were conducted by the U. S. Department of Agriculture (USDA), Aquatic Plant Management Laboratory, Fort Lauderdale, Florida. The following objectives were set forth: (a) test various formulations of 2,4-D and compare their relative toxicity to waterhyacinth, (b) determine the optimum rate and dilution at which herbicides should be applied, (c) determine the effect on plant kill of adding certain wetting agents to the spray solution, and (d) compare the herbicidal responses of waterhyacinth. This study concluded that all 2,4-D formulations tested at rates of 4.48 to 8.96 kg/ha were effective with only minor differences among formulations. Therefore, it has been the policy of the Corps of Engineers to follow these rates of application.

6. Schultz conducted studies to determine the uptake and dissipation of the dimethylamine salt of 2,4-D (2,4-D DMA) in water, sediment, and fish.[7] His studies were conducted in 11 ponds located at three different geographical and ecological sites. Residues of the 2,4-D DMA declined to less than 0.005 mg/ℓ in samples taken 28 days after application in Florida and Georgia pond waters, and in the 56-day postapplication samples from Missouri pond waters. The highest residue found in sediment was 0.17 mg/kg in the first- and third-day samples taken from the Missouri pond which was treated at 8.96 kg/ha. Residues were never found to be higher than 0.5 mg/kg in sediment from the Florida and Georgia ponds.

7. Although some data relating to 2,4-D residue levels and

the fate of this herbicide after application are available, the Environmental Protection Agency (EPA) found insufficient information available to support registration of 2,4-D DMA for control of aquatic weeds in the slow-moving and quiescent waters of the southern states. In December 1973 the EPA ordered the discontinuance of the use of 2,4-D not specifically labeled for use in flowing waters. All spraying operations thereafter were made under a temporary permit granted by the EPA. Data on 2,4-D DMA residues following spraying operations were needed to show that such applications would provide a safety margin that was consistent with the water usage in the areas treated. The water uses in this region include recreational activities, sports and commercial fishing, irrigation, and use as a potable water source. The 2,4-D tolerance limit established by EPA for potable water is 0.1 mg/ℓ.[8] Practically all freshwater areas of Louisiana and of the South are potential sources of potable and/or irrigation water.[9]

Purpose and Scope

8. This research project was undertaken to provide additional data necessary for registration of 2,4-D DMA for use in aquatic weed control in the slow-moving streams and waters of the southern states. The experiments were designed to provide information on 2,4-D residues at various distances from the point of application and at various times after the herbicide was applied. This information can be used to determine whether 2,4-D DMA could be safely used in slow-moving waters that are sources of potable and/or irrigation water.

9. A rice irrigation system afforded the unique and ideal situation whereby six different canals having a common water source provided the plot areas for this study. Application rates of 4.48- and 8.96-kg acid equivalent 2,4-D DMA/ha were compared. These rates represent the X and 2X rates of 2,4-D DMA used in waterhyacinth control operations by the Corps of Engineers and cooperating agencies.

10. Water samples were taken at various time increments after application. By combining the variables of rate, time of sampling, and

distance from the application site, the experiment allowed the monitoring of 2,4-D DMA residues in a relatively controlled water system.

11. Bioassay studies were also conducted utilizing tomato and rice plants as test crops and exposing these to water samples from one of the treated canals. Information derived both from the quantitative analyses of water samples and from the bioassay studies provides a basis for assessing residue hazards related to field treatments where waters are used for potable or irrigation purposes. The bioassay was undertaken to determine whether this procedure could be used as a quick and simple test for the presence of phytotoxic levels of 2,4-D in irrigation water. Such a detection procedure might be useful in determining whether treated waters could be used safely for irrigation of sensitive crops.

PART II: STUDY TECHNIQUE

Site Selection

12. A system of rice irrigation canals owned and operated by the Southdown Corporation of Louisiana was chosen as the test area for the experiment. This particular canal system is between Milton and Kaplan, Louisiana. It was chosen because it provided a main canal which served as a common water source for the six lateral canals used as individual test streams. The main canal originates at Milton, and its water source is the Vermilion River (Figure 1). The Vermilion River originates in Lafayette Parish and flows through Vermilion Parish where it empties into Vermilion Bay.

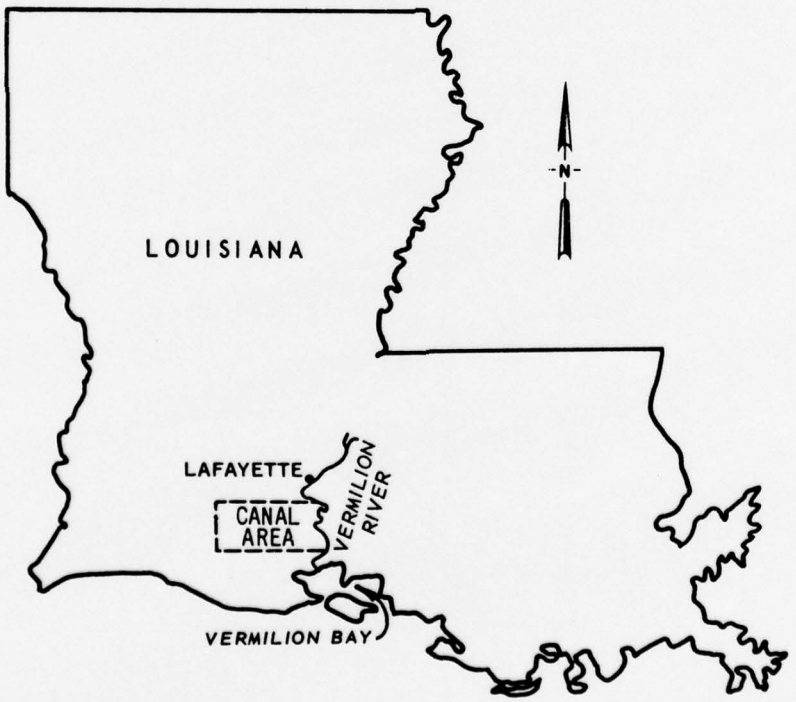

Figure 1. Vicinity map, location of the Vermilion River and the test canal area

13. The locations of the main canal and the six lateral canals used as test streams are shown in Figure 2. The land areas adjacent to the Vermilion River and to the canal system included the following major soil types: Jeanerette silt loam, Patoutville silt loam, Iberia clay, Beaumont clay, Midland silt loam, and Crowley silt loam. Rice and soybeans are the predominant crops grown in this area. Irrigation is standard procedure in rice production, but soybeans are seldom irrigated in this region.

14. The Vermilion River is characteristically turbid, as are most of the slow-moving streams in Louisiana. Colloidal silt particles account for most of the turbidity. Turbidity measurements for the Vermilion River at Lafayette, Louisiana, during 1974 ranged from a high of 110 mg/ℓ in May to a low of 30 mg/ℓ in August.[1] Levels of 2,4-D at the same location ranged from 0.1 µg/ℓ in July to 0.05 µg/ℓ in October 1974. These minute 2,4-D levels were considered negligible for the purpose of this experiment.

15. Each of the six lateral canals chosen as test streams extended for a distance of at least 6.4 km. The test canals are numbered 1 through 6 and their positions along the main canal are illustrated in Figure 2. Measurements characterizing water flow in the six test canals are presented in Table 1.

16. Surface velocity measurements made at the time of herbicide treatment varied between 0.1 m/sec for canals 1 and 2 and 0.3 m/sec for canals 5 and 6. These velocities were assumed satisfactory for classification as slow-moving water, since they fall within the range of average velocities for streams requiring treatment with 2,4-D in Louisiana and in other Gulf Coastal States. By comparing the test stream characteristics with those for streams in actual aquatic plant control areas, the data obtained in these experiments might be extrapolated to fit a variety of slow-moving stream situations. One possible variation of these irrigation canals from the natural stream profile is that most natural streams are not as deep along the edges and expose more sediment to the moving water.

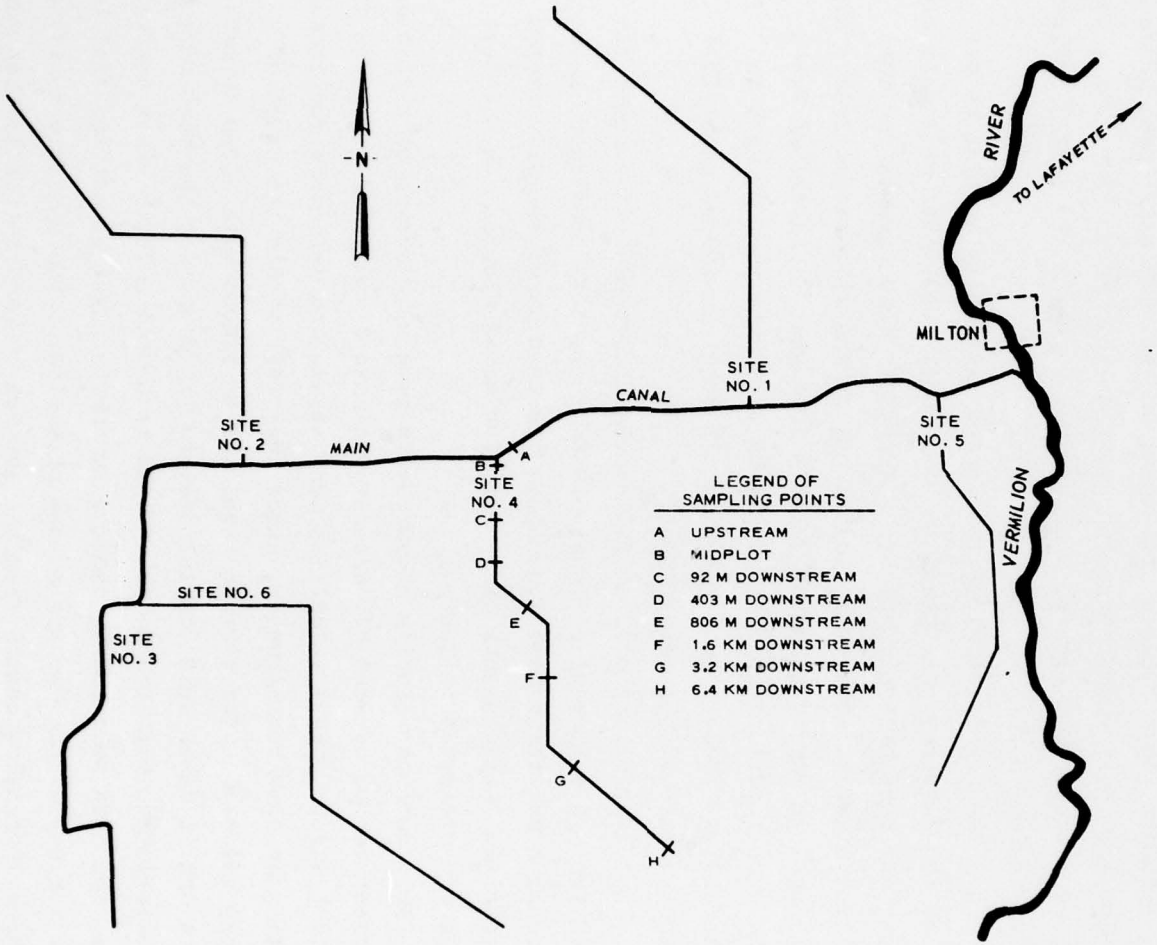

Figure 2. Location of the main canal, the lateral canals, and the test sampling sites

Experimental Procedure

Treatment application

17. The types of spray application used for control of waterhyacinth may vary from treatment of fringe areas along both banks to treatment of the entire surface area. The 2,4-D DMA treatments in canals 1, 2, 3, 4, and 6 were applied to a 3-m-wide by 166-m-long strip along both sides of these canals in order to simulate a fringe treatment. At these test sites the spray applications extended 0.6 m up the canal bank to control encroaching weeds and 2.4 m into the stream to control floating plants. The total treated area at these test sites was 0.1 ha. Rates of 2,4-D DMA equivalent to 4.48 kg acid equivalent/ha were applied at canal sites 1, 3, and 6, and rates equivalent to 8.96 kg acid equivalent/ha were applied at sites 2 and 4.

18. Application procedures at canal site 5 differed slightly from those described previously for the other test canals. At this site a 0.2-ha area was sprayed from bank to bank at a rate of 2,4-D DMA equivalent to 8.96 kg acid equivalent/ha. The entire spray volume at canal 5 was applied within the canal channel with no bank areas treated. This procedure simulated a treatment situation where aquatic vegetation covers the entire stream. In this type of application a greater concentration of the herbicide was actually applied over the water.

19. Although treatments were designed to simulate control of fringes of plant growth extending 3 m into the stream, at some points the fringe of weed growth was only about 1 m wide on each side. The predominant weeds included alligatorweed and waterhyacinth.

20. The herbicide application was made from a boat equipped with a power sprayer utilizing a handgun at 862 kPa. The volume of spray was equivalent to 950 ℓ/ha. Figure 3 shows this operation at canal 4.

Figure 3. Spray crew applying 2,4-D in the treated area at test canal 4

Water sampling

21. Water samples were taken from eight sites which varied in distance from the herbicide-treated zone (Figure 2). Sampling times for each site are given in Table 2. This schedule of sampling was used to provide data for the 2,4-D DMA residue time course study.

22. The samples consisted of a litre of water taken at a depth of 0.6 m and at a distance of 1.5 m from the bank. The samples were immediately acidified with 10 ml concentrated hydrochloric acid and refrigerated until analyses were performed. Procedures for extraction and gas chromatographic analyses were those outlined by Frank and Bartley[10] with the following modification. Prior to extraction, each water sample was filtered through Whatman No. 5 filter paper to remove clay particles suspended in the water.

Bioassay

23. A crude bioassay study was conducted to determine whether the 2,4-D in the water samples would produce detectable symptoms of

epinasty in tomato and rice seedlings. Tomato seedlings, cv. Venus, were grown in pots for 18 days and then watered over the top with 50 ml of water samples obtained from canal 3 at sampling sites and times indicated in Table 2. Standard solutions of 5.0, 1.0, 0.5, and 0.1 mg/ℓ 2,4-D DMA in tap water were prepared and applied to tomato plants in a manner similar to that described with canal water samples. The tomato plants were again watered over the top with the appropriate samples or standard solutions on the 19th day after seeding. Visual ratings for epinastic effects were made 2 weeks after the second treatment with the water samples. Ratings of epinasty were based on a scale where a 0 rating indicated no noticeable effect, 5 indicated moderate epinasty, and 10 indicated complete kill.

24. Rice seedlings, cv. Saturn, were grown and treated in a manner similar to that used for tomatoes. However, most of the rice seedlings were destroyed by a rainstorm shortly after treatment, and ratings for epinastic effects were of questionable reliability and are not reported herein.

Adsorption study

25. Because the canal waters contained considerable amounts of suspended silt particles, there was some question as to how much 2,4-D DMA might be adsorbed and lost from water samples in the process of filtration. In addition, sedimentation of silt particles could influence residues of 2,4-D in stream waters.

26. The objective of this study was to determine whether 2,4-D DMA applied to silt-laden canal water would be adsorbed to a significant extent, and whether filtration of water samples would reduce the 2,4-D recovered in analysis. This study was conducted in the laboratory and involved addition of 2,4-D DMA to canal water which contained 0.034, 0.068, and 0.136 g of silt per litre. The increased silt load was achieved by adding canal bottom sediment to canal water samples, contained in 2-litre beakers. The check consisted of 2,4-D DMA applied to distilled water. The treatments tested are listed as follows:

a. 2,4-D DMA at 0, 400, and 800 µg/ℓ in distilled water.

b. 2,4-D DMA at 0, 400, and 800 µg/ℓ in canal water containing 0.034 g silt per litre.

c. 2,4-D DMA at 0, 400, and 800 µg/ℓ in canal water containing 0.068 g silt per litre.

d. 2,4-D DMA at 0, 400, and 800 µg/ℓ in canal water containing 0.136 g silt per litre.

The 2,4-D DMA was added to the water samples at the rates indicated and allowed to interact at room temperature for 96 hr. Half of each sample was then filtered through Whatman No. 5 filter paper. Both the unfiltered and filtered fractions were extracted and analyzed for 2,4-D content.

Statistical Methods

27. The statistical design for the 2,4-D residue studies included a split-split plot with rate of applied 2,4-D as the whole plot, sampling site as the split plot, and time of sampling as the split-split plots. The experiment included two rates of applied 2,4-D, eight sampling sites, and thirteen sampling times. A total of six canals were included in the study. Canals 1 and 2, 3 and 4, and 5 and 6 were paired to form replicates 1, 2, and 3, respectively.

28. The use of this statistical design in analyzing the data is justified on the basis of the importance of the information desired. By inference, the higher the rate of applied 2,4-D, the greater the expected concentration at specified sampling times and sites. The primary purpose of this study was to determine the effect of time and distance on the concentration of 2,4-D in slow-moving canal water when 2,4-D is applied at some point upstream from the sampling sites.

29. A total of 624 water samples were collected over the duration of the study. However, in the interest of economy, only the selected samples indicated in Table 3 were extracted and analyzed for 2,4-D residues. Estimates of missing values for samples lost after collection were determined according to the procedures outlined by Cochran and Cox.[11] Analyses of variance were performed on residue data from sample sites A, B, and C. Sample site A was slightly upstream from the

point of 2,4-D application, site B was within the area where 2,4-D was sprayed, and site C was 92 m below the treated area. Figure 2 indicates the position of sampling sites used in this study at canal 4.

30. Sampling times included for each site were 1/2, 2, 8, and 48 hr after 2,4-D application. Mean concentrations for each time-rate treatment were compared using the t-test as outlined by Cochran and Cox.[11] A combined analysis of variance was also performed on the data from the three sites, and the mean 2,4-D concentrations at each site-time were compared. The analyses of variance for sites A, B, and C are shown in Appendix A.

31. Separate analyses of variance were conducted for sites D, E, F, G, and H using rate of applied 2,4-D and time of sampling as variables. The analyses of variance for these sites are also presented in Appendix A.

PART III: RESULTS OF STUDY

32. Analyses of variance were calculated on the individual sites labeled A, B, and C. Since site A was situated above the treated plot and received no 2,4-D, the only variable was sampling time. As expected, the analysis of variance indicated no significant differences in 2,4-D concentrations among canals and/or times of sampling for site A. This analysis of variance is presented in Appendix A. The mean 2,4-D concentration of 1.55 µg/ℓ shown in Table 4 for this site thus becomes a good estimate of 2,4-D concentration at any sampling time and reflects background levels in untreated canal water. The 2,4-D concentrations for each site and time are presented in Table 5, and the mean 2,4-D concentration for sites A, B, and C are presented in Table 4. The analysis of variance for site B, performed as that for a split-plot design with rate of applied 2,4-D as the whole plot and sampling time as the split plot, indicated no statistical significance for either variable. This analysis of variance is presented in Appendix A. Using the t-test for comparing means, all possible mean comparisons for site B were made. The test indicated no statistical significance among means. The mean 2,4-D concentrations were only slightly higher for site B than for site A as shown in Table 4.

33. Site C was analyzed in the same manner as site B and the results indicated no significance attributable to rate of applied 2,4-D or time of sampling. Comparisons among mean 2,4-D concentrations for site C indicated no statistical differences. The analysis of variance for site C is presented in Appendix A.

34. Of particular interest in reviewing the analysis of variance for the individual site was the small sum of squares attributable to rate of applied 2,4-D. Because these sums of squares were small and insignificant, rate of applied 2,4-D was not included in the combined analysis of variance.

35. In the combined analysis of variance shown in Appendix A, neither time of sampling nor sites were significant. This indicated that the 2,4-D concentration over time and distance was not statistically

different. The mean 2,4-D concentrations for sampling sites and times were calculated and all possible comparisons were made according to the aforementioned procedure. The results summarized in Table 4 indicate no statistically significant differences among the treatment means. The data in this table can also be interpreted to mean that within 1/2 hr after applying 2,4-D at site B, the mean 2,4-D concentrations at sites B and C were not statistically different from site A. Site A was located upstream from sites B and C and hence served as a check plot. The data obtained from the remaining sampling sites were analyzed by analysis of variance, and the results were similar to those obtained for sites A, B, and C. The analyses of variance for sites D, E, F, G, and H are given in Appendix A. Generally, the mean 2,4-D concentrations for sites downstream from site C were relatively small and it is doubtful that these concentrations would be statistically different from site A. Statistical comparison among all sites was not possible because different sampling times were selected for 2,4-D analysis at the downstream sites. It is logical to conclude from this study that the 2,4-D concentration in slow-moving canal water receiving applied 2,4-D will not increase significantly with time and distance from the point of application. As the water flows downstream, the applied 2,4-D apparently becomes diluted to the point that the mean concentration downstream is not measurably greater than the mean concentration above the treated site. The mean 2,4-D concentration for each sampling time (average of six canals) is graphically illustrated in Figure 4 and the mean 2,4-D concentration (average for all times and all canals) for sampling sites along a canal is illustrated in Figure 5.

36. At this point the following fundamental questions arise:

    a. Why were measurable levels of 2,4-D found in water upstream from the sprayed zone in each test canal?

    b. What happened to the 2,4-D applied in the sprayed zones?

37. The first question can be simply answered. The source water for these test canals was the Vermilion River which, as shown in Figure 1, flows through extensive agricultural areas. Background 2,4-D levels up to 0.1 µg/ℓ in Vermilion River water were mentioned earlier.

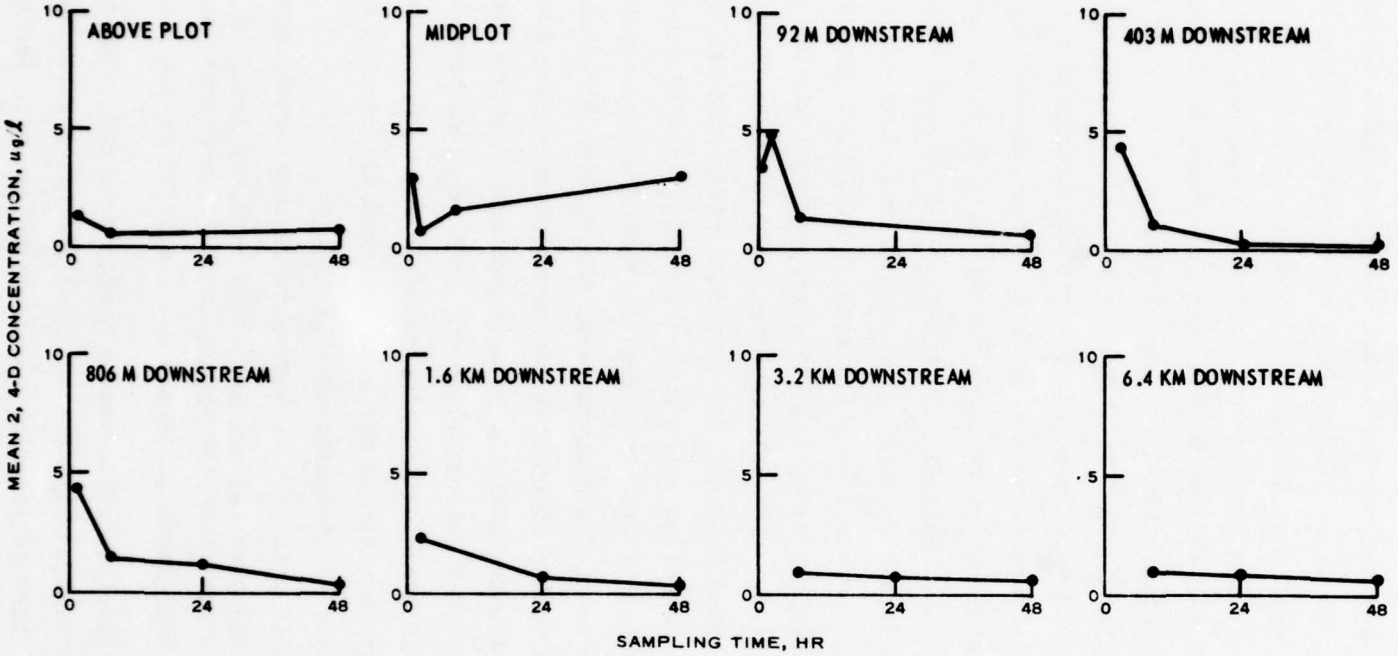

Figure 4. Mean 2,4-D concentration as a function of time for each sampling site

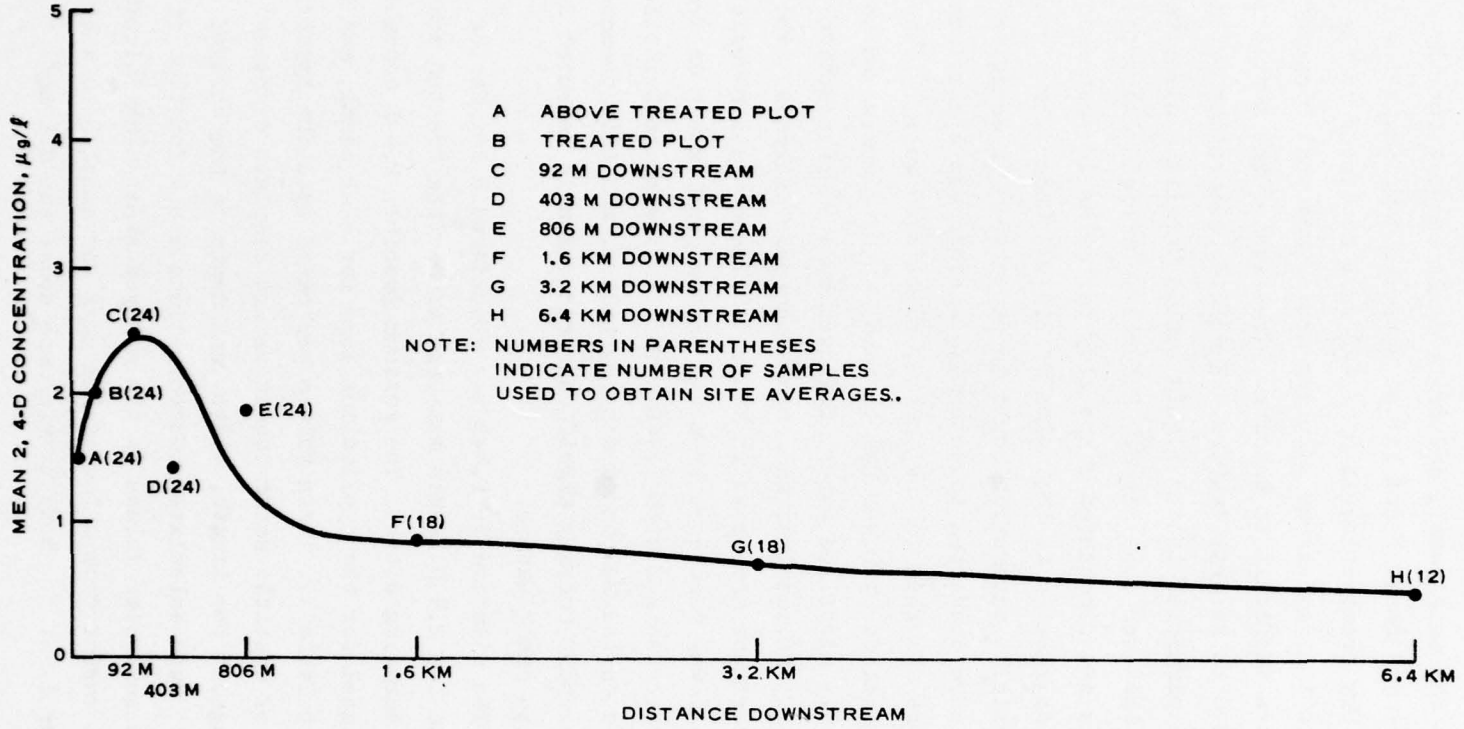

Figure 5. Mean 2,4-D concentrations as an average for all sampling times, locations, and canals

In addition, aerial applicators commonly spray rice fields that are adjacent to the main canal, and drift could account for the 2,4-D levels in the 1.55-µg/ℓ range found in the upstream sampling sites (Table 4).

38. Why greater levels of 2,4-D were not found in the sprayed zones and in the downstream sampling areas, and what happened to the 2,4-D is more difficult to resolve. The fate of the herbicide could be attributed to several factors. Herbicide adsorption on the suspended soil particles in the highly turbid Vermilion River waters was one factor that was considered. Extensive herbicide adsorption in these waters was discounted since clay particles in suspension are negatively charged as is the anion of 2,4-D. Under these conditions limited herbicide adsorption could be expected. In addition, laboratory tests were conducted to determine whether any significant amounts of 2,4-D were adsorbed in the turbid, silt-laden water. These tests involved application of 2,4-D DMA to canal water samples and to samples including only distilled water, followed by a 96-hr reaction period, filtration of the samples, and chromatographic analysis. The results of this experiment are shown in Table 6. These tests revealed that as much 2,4-D was recovered from the turbid canal water as from the distilled water spiked with 2,4-D. This indicated that little 2,4-D was adsorbed on the suspended clay particles, and this phenomenon could not account for any significant reduction in measured 2,4-D levels in the canal waters.

39. The dilution of 2,4-D was considered a key factor in explaining the low 2,4-D levels measured within the treated area and in downstream sampling sites. The maximum possible 2,4-D concentrations were calculated for the application zone for each canal, and these are presented in Table 1. These values are based upon the hypothetical assumption of a static water condition and complete dispersal of applied 2,4-D throughout the length, width, and depth of the sprayed zone for each canal. Such calculated concentrations are a function of rate of application and water volume in the sprayed zone. The calculated concentrations varied from a low of 118 µg/ℓ for canal 3 to a high of 818 µg/ℓ for canal 5. Both levels were above the 0.1-mg/ℓ tolerance for

potable waters established by the EPA. It would seem logical that the 2,4-D levels obtained by analysis should have been higher at sites in canal 5 than for comparable sites in canal 3 (Table 5); however, no differences were found.

40. One cannot assume complete dispersal of the 2,4-D residues under any lake or stream conditions. The sprayed 2,4-D that reaches the water surface probably moves slowly into the main stream and then away from the treated vegetation. In addition, the depth of sampling could be an important factor. It would seem logical that surface water would contain more residue than samples drawn from the bottom of the stream shortly after application. Samples in this study were obtained from a depth of 0.6 m at a distance of 1.5 m from the canal bank. Normally potable water or irrigation intake lines will be at least this deep.

41. As mentioned previously, water in this entire irrigation system contained varying amounts of 2,4-D during the late spring and summer. It is reasonable to assume that the presence of this chemical will maintain a population of microorganisms which biodegrade some of the 2,4-D, thereby accounting for part of the herbicide loss.

42. The most logical explanation of the low levels of 2,4-D recovered lies in the fact that the object of spraying in the first place is to cover undesirable aquatic vegetation both floating and that encroaching from the bank. If this is done with any degree of efficiency most of the applied 2,4-D is not instantly injected into the water. In fact, much of the herbicide that contacts the plant may be photodegraded, or biodegraded, and may never enter the water. The 2,4-D remaining on the plant and that translocated into the plants will not come into contact with the stream waters until the plants sink and decompose some time after application. For senescence and decomposition to begin, a time lapse of perhaps 4 days to a full month may be involved.

43. After consideration of the above discussion it is illogical to assume that all the applied 2,4-D enters the water column at one time. On the contrary, it appears that following careful and thorough herbicide applications, small levels of herbicide will be present in

the water at any given time. In addition, any significant level of herbicide accumulation is further prevented by the slow but continuous stream movement away from the treated zone. The results of the residue analyses presented in Tables 4 and 5 and in Figures 4 and 5 bear out these observations.

44. Table 5 shows that none of the samples analyzed either from sample site B or from downstream sites approached the theoretical concentrations indicated in Table 1. It should be noted in Table 5 that the concentrations of 2,4-D detected in some samples collected above the plots exceeded levels collected within and below the treated plots. It is reasonable to assume that the applied 2,4-D was greatly diluted and transported downstream by the slow-moving waters. From the previous discussion it may be seen that the analyses of water samples showed no significant differences in 2,4-D concentrations among the various canals and/or times of sampling for the individual sites A, B, and C. The combined analysis of variance also indicated no significant differences among canals, sites, and times of sampling.

45. It was anticipated that the level of 2,4-D concentrations at the 1/2-hr sampling time at site B would be considerably greater than for other sampling times. However, the comparisons in Table 4 indicate that within 1/2 hr after spraying the mean concentration of 2,4-D was not significantly different from that above the sprayed zone or downstream from the sprayed zone.

46. In 1975 large-scale applications of 2,4-D DMA for waterhyacinth control on the St. Johns River were monitored by Joyce and Sikka[12] and their results verify the findings reported herein. They found 2,4-D levels ranged from nondetectable to 1.3 µg/ℓ following spraying and reported no apparent correlation between quantities of 2,4-D applied and the residues detected in the water.

47. Results of the bioassay study with tomatoes are shown in Table 7. No apparent differences were found between the assays for the three sampling sites (A, B, and C). This agrees with the analytical data for these same sites. Although there was some agreement between the bioassay and the chemical analyses, it is apparent that the bioassay

techniques employed were not consistently sensitive enough to indicate the low 2,4-D levels involved. The tomato plants treated with prepared standards of 2,4-D DMA in distilled water ranging from 0.1 to 5.0 mg/ℓ did show pronounced symptoms of epinasty. This indicates that such an assay may not be sensitive in the µg/ℓ range of concentrations, but may be used as a qualitative and perhaps a crude quantitative test for 2,4-D residues at higher levels of concentration.

PART IV: CONCLUSIONS AND RECOMMENDATIONS

48. The data presented show that 2,4-D residues were always well below the 0.1-mg/ℓ level established for potable water by the EPA at all sampling points in the six test canals regardless of time after application. The low concentrations of measured 2,4-D residues at all sampling points and times are attributed principally to the dilution of the herbicide when applied under actual control situations for floating aquatic species such as waterhyacinth. Adsorption of 2,4-D to suspended clay particles in the treated water was found to be minimal in this study.

49. Results similar to these could be expected under actual field application situations. Measured residue levels where 2,4-D is applied at comparable rates and under similar conditions would be expected to be well within established tolerances for potable water supplies and for irrigation waters.

50. Bioassay studies with rice and tomato seedlings showed this procedure was not sensitive enough to consistently detect low 2,4-D residues in the canal waters. Tomato plants did show epinasty symptoms when treated with prepared standard 2,4-D mixtures in the 0.1- to 5-mg/ℓ range. This bioassay procedure cannot be used as a quantitative test but could perhaps be used qualitatively as a test for presence of 2,4-D at levels of 1 mg/ℓ or above.

51. As a result of these studies the following recommendations are suggested:

a. That applications of 2,4-D DMA for control of floating aquatic species at rates currently labeled be continued in the slow-moving and quiescent waters of the South.

b. That periodic monitoring of spray programs be conducted by the Corps of Engineers and others involved in control programs utilizing 2,4-D DMA. Data resulting from such a monitoring program would provide residue information to applicators allowing them to adjust spray operations to avoid excessive 2,4-D residues, thereby affording maximum safety to man, his agricultural crops, and the environment.

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Table 1

Streamflow Characteristics and Calculated

2,4-D Concentration Levels

Canal	Cross-Sectional Area,* m^2	Surface Velocity m/sec	Average Stream Velocity** m/sec	Flow Rate† m^3/sec	Calculated 2,4-D Concentration†† µg/ℓ
1	17.48	0.10	0.060	1.06	157
2	22.99	0.09	0.055	1.28	240
3	23.39	0.17	0.100	2.39	118
4	14.73	0.18	0.106	1.56	374
5	11.07	0.31	0.182	2.02	818
6	13.66	0.32	0.182	2.49	201

* Area was computed by using an average of three planimeter readings.

** Average stream velocity was computed by using V_{avg} = (0.6) × average surface velocity.

† Flow rate was computed by using Q = cross-sectional area × average stream velocity.

†† Calculation of 2,4-D concentration was based upon water volume in the treated and channel area of each site at the appropriate treatment rate and assuming a static water condition.

Table 2

Sampling Site Location and Times of Sampling

Sampling Station		
Designation	Location	Time of Sampling
A	Upstream from treated area	Before treatment and 0.5, 1, 2, 4, 8, 16, 24 hr, and 2, 4, 8, 16, and 32 days after treatment
B	Middle of the treated zone	Same as above
C	92 m below treated zone	Same as above
D	403 m below treated zone	First sampling at 1 hr, otherwise same as above
E	806 m below treated zone	First sampling at 1 hr, otherwise same as above
F	1.6 km below treated zone	First sampling at 2 hr, otherwise same as above
G	3.2 km below treated zone	First sampling at 4 hr, otherwise same as above
H	6.4 km below treated zone	First sampling at 8 hr, otherwise same as above

Table 3

Samples Selected for 2,4-D Analysis

Time After 2,4-D DMA Application, hr	Sampling Sites							
	A	B	C	D	E	F	G	H
1/2	X	X	X					
2	X	X	X	X	X	X		
8	X	X	X	X	X	*	X	
24				X	X	X	X	X
48	X	X	X	X	X	X	X	X

* Samples for site F at 8 hr were inadvertently omitted.

Table 4

Mean 2,4-D Residues at Sampling Sites A, B, and C

Sampling Times, hr	2,4-D Concentration, µg/ℓ			
	Site A	Site B	Site C	Mean
1/2	3.33	2.89	3.42	3.21
2	1.45	0.61	4.90	2.32
8	0.70	1.47	1.25	1.14
48	0.71	3.02	0.60	1.45
Mean*	1.55	2.00	2.54	2.03

* The means were compared according to the procedures outlined by Cochran & Cox,[11] and no significant differences were found to exist among sites, among times within a site, and among times at different sites.

Table 5

Results of 2,4-D Residue Analyses for Selected Sampling Sites and Times

Sampling Sites and Time After Application		Concentration, µg/ℓ, of 2,4-D in Canal						
Site	Time, hr	1	2	3	4	5	6	Mean
A	1/2	1.42	0.38	14.81	2.07	1.32	0.00	3.33
A	2	2.87	0.27	1.03	2.62	1.56	0.32	1.45
A	8	2.05	0.30	1.13	0.35	0.00	0.34	0.70
A	48	0.57	0.74	0.35	2.60	0.00	0.00	0.71
B	1/2	10.41	0.30	3.46	1.62	0.20	1.32	2.90
B	2	0.25	0.17	1.39	1.49	0.15	0.23	0.61
B	8	1.76	1.54	0.99	2.95	0.13	1.43	1.47
B	48	15.81	0.95	0.19	1.13	0.00	0.10	3.03
C	1/2	2.13	0.00	0.11	9.98	7.25	1.09	3.43
C	2	9.16	1.34	4.90	13.74	0.16	0.11	4.90
C	8	0.70	0.24	1.23	3.30	0.00	2.02	1.25
C	48	0.00	0.45	0.51	2.60	0.00	0.01	0.60
D	2	2.71	8.80	0.17	11.76	0.09	4.06	4.60
D	8	2.24	0.00	1.52	2.58	0.09	0.00	1.07
D	24	0.59	0.10	0.00	0.49	0.05	0.00	0.21
D	48	0.08	0.11	0.17	0.59	0.08	0.58	0.27
E	2	4.83	3.94	2.73	8.54	0.98	7.15	4.70
E	8	3.82	0.09	2.09	2.78	0.34	0.17	1.55
E	24	0.08	0.02	5.32	1.72	0.00	0.90	1.34
E	48	0.06	0.17	0.05	0.62	0.00	0.06	0.16
F	2	0.94	0.00	0.00	0.88	0.00	10.39	1.74
F	24	0.53	0.00	1.04	0.33	1.03	0.00	0.49
F	48	0.00	0.28	0.13	0.80	0.18	0.00	0.24
G	8	1.33	0.00	1.32	0.22	1.04	1.27	0.87
G	24	0.46	0.00	0.28	2.56	0.60	0.16	0.68
G	48	1.83	0.50	0.00	1.37	0.10	0.08	0.65
H	24	0.00	3.63	0.12	0.17	0.44	0.00	0.73
H	48	0.00	0.15	0.00	2.46	0.00	0.00	0.44
Mean		2.38	0.87	1.61	2.94	0.57	1.14	

Table 6

Effect of Silt Content in Canal Water upon 2,4-D Recovery by Gas Chromatographic Analysis

Water Source	2,4-D Concentration µg/ℓ	2,4-D Recovered in Analysis,* µg/ℓ		
		Filtered	Unfiltered	Mean
A. Distilled water	0	2.5	2.5	2.5
	400	187.0	187.0	187.0
	800	548.0	548.0	548.0
B. Canal water with 0.034 g silt/litre	0	15.0	18.0	17.0
	400	237.0	200.0	237.0
	800	321.0	350.0	336.0
C. Canal water with 0.068 g silt/litre	0	10.0	8.0	9.0
	400	284.0	271.0	266.0
	800	627.0	486.0	507.0
D. Canal water with 0.136 g silt/litre	0	2.5	2.5	2.5
	400	332.0	201.0	267.0
	800	615.0	642.0	629.0

* Lowest detectable level of 2,4-D is 2.5 µg/ℓ.

Table 7

Rating of Epinasty in Tomato Plants Treated with Water Samples from Canal 3 and with Standard 2,4-D Preparations

Sample Site Location	Visual Ratings of Epinasty* at Times of Sample Collection After Treatment							
	0 hr	1/2 hr	1 hr	2 hr	4 hr	6 hr	16 hr	Mean
A, above plot	0.0	1.0	1.0	1.3	1.0	0.7	2.0	1.0
B, midplot	0.7	0.0	0.7	1.7	0.3	1.3	1.3	0.9
C, 92 m below plot	0.0	0.3	1.0	1.3	0.7	1.0	1.0	0.8
Mean	0.2	0.4	0.9	1.4	0.7	1.0	1.4	

Concentration, µg/ℓ of 2,4-D in Standard Preparations	Visual Ratings of Epinasty*
0.0 (tap water)	0.3
0.1	2.7
0.5	4.3
1.0	3.7
5.0	6.3

* Ratings: 0 = no visible symptoms of epinasty, 5 = moderate epinasty, and 10 = complete kill of tomatoes.

APPENDIX A: ANALYSES OF VARIANCE CONDUCTED FOR THE VARIOUS SAMPLING SITES

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	F.05
Analysis of Variance--Site A					
Total	35	202.51			
Replicates (canals)	5	47.07	9.41	1.11	2.90
Times	3	27.77	9.26	1.09	3.29
Error	15	127.67	8.51		
Analysis of Variance--Site B					
Total	23	302.67			
Replicates	2	49.15	24.57	0.96	19.90
Rate (R)	1	29.73	29.73	1.16	18.51
Error (a)	2	51.11	25.55		
Time (T)	3	24.31	8.10	<1	3.71
R × T	3	31.61	10.53	<1	3.71
Error (b)*	10	116.71	11.67		
Analysis of Variance--Site C					
Total	23	325.50			
Replicates	2	48.93	24.46	<1	19.00
Rates (R)	1	12.17	12.17	<1	18.51
Error (a)	2	67.74	33.87		
Time (T)	3	70.89	23.63	2.26	3.71
R × T	3	21.32	7.11	<1	3.71
Error (b)*	10	104.45	10.44		
Combined Analysis of Variance--Sites A, B, and C					
Total	71	824.64			
Replicates (canals)	5	146.23	29.24	1.83	3.33
Site (s)	2	11.96	5.98	<1	4.10
Error (a)	10	159.67	15.97		
Time (T)	3	47.54	15.85	1.62	2.84
R × T	6	75.43	12.57	1.28	2.33
Error (b)**	41	401.81	9.80		
Analysis of Variance--Site D					
Total	23	198.32			
Replicates	2	10.63	5.31	<1	19.00
Rate (R)	1	6.27	6.27	<1	18.51
Error (a)	2	20.40	10.20		
Time (T)	3	70.01	23.33	3.52	3.71
R × T	3	24.84	8.28	1.25	
Error (b)*	10	66.17	6.61		

* Degrees of freedom for error (b) reduced by 2 because of missing values.
** Degrees of freedom for error (b) reduced by 4 because of missing values.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F	F.05
	Analysis of Variance--Site E				
Total	23	140.72			
Replicates	2	13.87	6.93	1.99	19.00
Rates (R)	1	2.71	2.71	<1	18.51
Error (a)	2	7.48	3.49		
Time (T)	3	62.63	20.87	3.64	3.71
R × T	3	2.45	.0.82	<1	3.71
Error (b)*	9	51.58	5.73		
	Analysis of Variance--Site F				
Total	17	97.86			
Replicates	2	9.44	4.72	<1	19.00
Rate (R)	1	2.26	2.26	<1	18.51
Error (a)	2	12.14	6.07		
Time (T)	2	11.42	5.71	<1	4.46
R × T	2	16.16	8.08	1.39	4.46
Error (b)	8	46.44	5.81		
	Analysis of Variance--Site G				
Total	17	9.42			
Replicates	2	0.68	0.34	<1	19.00
Rate (R)	1	0.15	0.15	<1	18.51
Error (a)	2	3.25	1.62		
Time (T)	2	0.17	0.08	<1	4.46
R × T	2	1.88	0.94	2.29	4.46
Error (b)	8	3.29	0.41		
	Analysis of Variance--Site H				
Total	11	15.54			
Replicates	2	1.46	0.73	<1	19.00
Rates (R)	1	3.77	3.77	4.90	18.51
Error (a)	2	1.53	0.77		
Time	1	0.08	0.08	<1	7.71
R × T	1	4.42	4.42	4.21	7.71
Error (b)	4	4.19	1.05		

* Degrees of freedom for error (b) reduced by 3 because of missing values.

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